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The Effect of a 3-Month Low-Intensity Endurance Training Program on Fat Oxidation and Acetyl-CoA Carboxylase-2 Expression

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Endurance training has been shown to increase fat oxidation both at rest and during exercise. However, most exercise training studies have been performed at high exercise intensity in well-trained athletes, and not much is known about the effect of a low-intensity training program on fat oxidation capacity in lean sedentary humans. Here, we examine the effect of 3-month low-intensity training program on total and intramuscular triglyceride (IMTG)- and/or VLDL-derived fat oxidation capacity and skeletal muscle mRNA expression. Six healthy untrained subjects (aged 43 ± 2 years, BMI 22.7 ± 1.1 kg/m^2, Vo_{2max} 3.2 ± 0.2 l/min) participated in a supervised 12-week training program at 40% Vo_{2max} three times weekly. Total and plasma-derived fatty acid oxidation at rest and during 1 h exercise was measured using [^{13}C]palmitate, and in a separate test, [^{13}C]acetate recovery was determined. Muscle biopsies were taken after an overnight fast. Total fat oxidation during exercise increased from 1,241 ± 93 to 1,591 ± 130 μmol/min (P = 0.06), and IMTG- and/or VLDL-derived fatty acid oxidation increased from 236 ± 84 to 639 ± 172 μmol/min (P = 0.09). Acetyl-CoA carboxylase-2 mRNA expression was significantly decreased after training (P = 0.005), whereas lipoprotein lipase mRNA expression tended to increase (P = 0.07). In conclusion, a minimal amount of physical activity tends to increase fat oxidation and leads to marked changes in the expression of genes encoding for key enzymes in fat metabolism. *Diabetes* 51:2220–2226, 2002

The prevalence of obesity and type 2 diabetes is rapidly increasing in Western societies. The consumption of high-fat diets and a gradual decrease over the last century in voluntary physical activity plays an important role in the increase of the prevalence of both conditions (1,2). Humans exhibit a large interindividual difference in their capacity to oxidize fat, and low rates of fat oxidation have been associated with accelerated weight gain in Pima Indians (3). Several studies, though not all, have observed low rates of fat oxidation in obese and type 2 diabetic subjects both at rest and during exercise (4,5). In humans, the following fat sources are available for oxidation: *1*) plasma fatty acids, released from lipolysis of adipose tissue triglycerides into the blood, represent the major source; *2*) fatty acids released from circulating VLDL triglycerides by the action of lipoprotein lipase (LPL); and *3*) intramuscular triglyceride (IMTG) depots. A diminished capacity to oxidize fat will result in the storage of fat in adipose tissue as well as inside muscle cells. Indeed, obesity and type 2 diabetes are characterized by accumulation of fat into IMTG depots (6), and importantly, these triglyceride depots are negatively associated with insulin action (7). Thus, prolonged inhibition of carnitine-palmitoyl-transferase-1 (CPT1), the rate-limiting enzyme in fat oxidation, using etomoxir results in accumulation of IMTGs and insulin resistance (8). Therefore, a low fat oxidation capacity, in combination with a Western (high calorie and high fat) diet, could be one of the factors predisposing to insulin resistance. One way to increase the rates of fat oxidation is by means of endurance training (9). Endurance-trained athletes oxidize more fat than untrained subjects, both at rest and during exercise (10–13). Therefore, endurance training could possibly be a way to overcome a low fat oxidative capacity. However, in most of the training studies, exercise was performed at moderate to high intensities for many hours per week and such regimens may not be applicable for the prevention and/or treatment of obesity and type 2 diabetes. An unanswered question is whether regular physical activity at low intensity and for only a few hours weekly is also able to increase fat oxidation. To address this question, we designed a study that examined the effect of 3 months of low-intensity physical activity three times weekly in lean sedentary humans.

Considering the relationship between IMTGs and insulin resistance, it is of particular interest whether endurance training is able to increase the oxidation of these lipid stores; however, available data are conflicting. Some studies have reported an increased use of IMTG- and/or VLDL-derived fatty acids by endurance training (12,14–16), whereas others did not observe such an increase (17,18). Part of this controversy might be explained by methodological problems to examine the relative contribution of the different fat sources to total fat oxidation

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ACC2, acetyl-CoA carboxylase-2; CPT1, carnitine-palmitoyl-transferase-1; FFA, free fatty acid; GC, gas chromatography; IMTG, intramuscular triglyceride; IR, isotope ratio; LPL, lipoprotein lipase; MS, mass spectrometry; TCA, tricarboxylic acid; TTR, tracer/tracee ratio; UCP3, uncoupling protein-3.

TABLE 1
Subject characteristics

Subject	Before	After	P
Age (years)	42.7 ± 2.0	—	
Height (m)	1.78 ± 0.02	—	
Weight (kg)	71.4 ± 2.8	70.3 ± 2.9	0.07
Body fat (%)	18 ± 2	17 ± 2	0.50
BMI (kg/m^2)	22.7 ± 1.0	22.3 ± 0.9	0.07
Vo_{2max} (l/min)	3.2 ± 0.2	3.3 ± 0.1	0.57

Data are means ± SE.

during exercise. The biochemical determination of IMTG content of the skeletal muscle is known to be problematic. On the other hand, determination of plasma-derived fatty acid oxidation using stable isotope tracers has long been questioned, and only since the introduction of the acetate recovery factor in 1995 (19) could the oxidation of labeled fatty acids be reliably determined (20,21). Therefore, the second aim of this study was to use this methodology to resolve the controversy whether endurance training is able to increase the capacity to oxidize IMTG- and/or VLDL-derived fatty acids.

The molecular adaptation of skeletal muscle to low-intensity endurance training is largely unknown. GLUT4, the major glucose transporter in skeletal muscle, and hexokinase II, which catalyzes the phosphorylation of glucose to glucose-6-phosphate, are two key genes involved in glucose utilization. Considering that endurance training is able to increase IMTG- and/or VLDL-derived fatty acid oxidation, LPL and acetyl-CoA carboxylase-2 (ACC2) are two key genes that are possibly involved in this adaptation to endurance training. LPL is responsible for hydrolysis of plasma triglycerides and directs the released free fatty acids (FFAs) into the tissue (22). Inside the muscle cell, ACC2 has recently been suggested to control the rate of fatty acid oxidation and triglyceride storage (23). Finally, the skeletal muscle–specific uncoupling protein-3 (UCP3) has also been suggested to be involved in fatty acid metabolism, but the exact function is still under debate (24). Therefore, the third aim of the present study was to examine the effect of low-intensity endurance training on the expression of the above-mentioned genes.

RESEARCH DESIGN AND METHODS

Subjects. The characteristics of the six healthy nonobese male volunteers are presented in Table 1. None of the subjects spent >2 h per week in sports activities or had physically demanding jobs. The nature and risks of the experimental procedure were explained to the subjects, and all subjects gave written informed consent. The study was approved by the Medical-Ethical Committee of Maastricht University.

Experimental design. Subjects participated in two stable isotope experiments, separated by 1 week, to measure total and plasma-derived fatty acid oxidation in random order. In these tests, an infusion of either [U-^{13}C]palmitate or [1,2-^{13}C]acetate was given for 2 h at rest and 1 h during exercise. Acetate is directly converted to acetyl-CoA, and the recovery of acetate can be used to correct [^{13}C]palmitate oxidation for loss of label in the tricarboxylic acid (TCA) cycle, as previously described (19,25). On a separate day, a muscle biopsy was taken after an overnight fast. Immediately after the last stable isotope experiment, the training period was started. After the 12-week training program, a second muscle biopsy was taken 6–7 days after the last training session. The two stable isotopes experiments were repeated 7–8 and 14–15 days after the end of the 12-week training program in random order. In this way, it was prevented that the last training session could influence the measurements. Three days before the first stable isotope experiment, subjects were asked to write down their food intake and to consume the same food items before every other stable isotope experiment. Subjects were asked not to consume any products with a high abundance of ^{13}C (carbohydrates derived from C4 plants such as maize and sugar cane) 1 week before and during the entire experimental period. Subjects were asked to refrain from physical activity 2 days before the sampling of the muscle biopsy and before the stable isotope experiments.

Training program. The exercise training program consisted of cycling on an ergometer (Bodyguard Cardiocycle, Sandnes, Norway or Lode, Groningen, the Netherlands) at a low intensity (40% of predetermined Vo_{2max}). Subjects trained three times per week for 12 weeks. Energy expenditure of each subject in each training session was 5 kcal/kg fat-free mass (280–300 kcal). Training duration for subjects per session was 47.5 ± 2.5 min. Heart rate was monitored continuously during the training sessions (Polar Electro, Oy, Finland). After 4 and 8 weeks of exercise training, a maximal aerobic exercise test was performed, and the training workload and duration were adjusted if necessary. All training sessions took place at the university under the supervision of a professional trainer.

Procedures

Body composition. One week before and after the training program, body density was determined by underwater weighing in the fasted state. Body weight was measured with a digital balance, accurate to 0.01 kg (type E1200; Sauter). Lung volume was measured simultaneously with the helium dilution technique using a spirometer (Volugraph 2000; Mijnhardt). Body fat percentage was calculated using the equations of Siri (26). Fat-free mass, in kilograms, was calculated by subtracting fat mass from total body mass.

Maximal aerobic capacity. One week before and after the training program, each subject performed an incremental exercise test on an electronically braked cycle ergometer (Lode Excalibur) to determine maximal oxygen consumption (Vo_{2max}) and maximal power output (W_{max}). Exercise was performed until voluntary exhaustion or until the subject could no longer maintain a pedaling rate ≥60 rpm. Subjects started cycling at 75 W for 5 min. Thereafter, workload was increased by 50 W every 2.5 min. When subjects were approaching exhaustion, as indicated by heart rate and subjective scoring, the increment was reduced to 25 W. Heart rate was registered continuously using a Polar Sport tester (Kempele, Finland). Oxygen consumption and carbon dioxide production were measured using open circuit spirometry (Oxycon-β; Mijnhardt).

Isotope infusion. At 8:00 A.M. after an overnight fast, subjects underwent an isotope infusion test. Teflon catheters were inserted in an antecubital vein for isotope infusion and retrogradely into a contralateral dorsal hand vein for sampling of arterialized venous blood. After placement of the catheters, subjects rested on a bed, and the cannulated hand was placed in a hotbox, in which air was circulated at 60°C to obtain arterialized venous blood. After 30 min, baseline oxygen consumption and carbon dioxide production was measured, and breath and blood samples were collected. Immediately thereafter, subjects were given an intravenous dose of 0.085 mg/kg $NaH^{13}CO_3$ to prime the bicarbonate pool. Then, at time zero, a constant intravenous infusion of either [U-^{13}C]palmitate (0.00806 μmol · min^{-1} · kg^{-1}) or [1,2-^{13}C]acetate (0.0645 μmol · min^{-1} · kg^{-1}) was started and continued for 120 min at rest. With these infusion rates, the amount of ^{13}C infused during palmitate and acetate infusion are similar. After the 120-min rest period, subjects cycled at 50% W_{max} for 1 h, and the infusion rate of palmitate or acetate was doubled to ensure sufficient ^{13}C plasma palmitate enrichment. Blood samples and breath samples were taken at 0, 100, 110, and 120 min at rest and 160, 170, and 180 min during exercise. At rest, Vo_2 and VCO_2 were measured continuously during the first 90 min using open circuit spirometry (Oxycon-β). During exercise, Vo_2 and VCO_2 were measured immediately before the measurement of breath $^{13}CO_2$ enrichment.

Isotope preparations. To determine the exact infusion rate, the concentration of palmitate in the infusate was measured for each experiment using analytical gas chromatography (GC) using heptadecanoic acid as internal standard (see sample analysis). The palmitate tracer (60 mg potassium salt of [U-^{13}C]palmitate, 99% enriched; Cambridge Isotope Laboratories, Andover, MA) was dissolved in heated sterile water and passed through a 0.2 μm filter into a 5% warm human serum albumin to make a 0.670 mmol/l solution. The acetate concentration was measured in each infusate with an enzymatic method (Boehringer Mannheim, Mannheim, Germany). The acetate tracer (sodium salt of [1,2-^{13}C]acetate, 99% enriched; Cambridge Isotope Laboratories) was dissolved in 0.9% saline. The chemical and isotopic purity (99%) of palmitate and acetate tracers were checked by ^{1}H and ^{13}C NMR (nuclear magnetic resonance) and GC/mass spectrometry (MS).

Muscle biopsy sampling and analysis. Muscle biopsies were taken from the mid-thigh region from *M. vastus lateralis* according to the technique of Bergstrom et al. (27) in five of six subjects 6–7 days before or after the training program. The subjects were required to abstain from training or vigorous exercise 48 h before the biopsy. The biopsy was used for isolation of total RNA using the acid phenol method of Chomozynski and Sacchi (28), with an

TABLE 2
Palmitate and breath CO_2 enrichment before and after training

Time (min)	[^{13}C]palmitate enrichment (TTR × 1,000)		Breath $^{13}CO_2$ enrichment (TTR × 1,000)	
	Before	After	Before	After
100	4.134 ± 0.473	5.471 ± 1.293	0.083 ± 0.002	0.075 ± 0.004
110	3.896 ± 0.451	5.116 ± 1.023	0.088 ± 0.003	0.080 ± 0.005
120	4.405 ± 0.426	5.99 ± 1.438	0.096 ± 0.003	0.085 ± 0.006
160	3.805 ± 0.308	5.435 ± 1.326	0.159 ± 0.005	0.159 ± 0.010
170	3.719 ± 0.315	3.771 ± 0.911	0.164 ± 0.004	0.162 ± 0.010
180	3.511 ± 0.270	4.192 ± 0.764	0.170 ± 0.003	0.166 ± 0.011

Data are means ± SE.

additional DNAse digestion step with concomitant acid phenol extraction and ethanol precipitation. The mRNA levels of LPL, hexokinase II, GLUT4, ACC2, and UCP3 were quantified by RT-competitive PCR (29). For the assays, the RT reaction was performed from 0.1 μg skeletal muscle total RNA in the presence of a thermostable reverse transcriptase (Tth; Promega) by use of one of the specific antisense primers. The competitive PCR assays were performed as previously described (30–32). To improve the quantification of the amplified products, fluorescent dye–labeled sense oligonucleotides were used. The PCR products were separated and analyzed on an ALFexpress DNA sequencer (Pharmacia) with the Fragment Manager Software. Total RNA preparations and RT-competitive PCR assays of the two skeletal muscle samples from the same individual (before and after weight loss) were performed simultaneously.

Plasma and expired air analysis. Oxygen saturation (Hemoximeter OSM2; Radiometer, Copenhagen, Denmark) was determined immediately after sampling in heparinized blood and used to check arterialization. Fifteen milliliters of arterialized venous blood was sampled in tubes containing EDTA to prevent clotting and immediately centrifuged at 3,000 rpm (1,000*g*) for 10 min at 4°C. Plasma was immediately frozen in liquid nitrogen and stored at −80°C until further analysis. Plasma substrates were determined using the hexokinase method (Roche, Basel) for glucose, the Wako NEFA (nonesterified fatty acid) C test kit (Wako Chemicals, Neuss, Germany) for FFAs, and the glycerolkinase-lipase method (Boehringer Mannheim) for glycerol and triglycerides.

Breath samples were analyzed for $^{13}C/^{12}C$ ratio using a GC-isotope ratio (IR) MS system (GC-IRMS) (Finnigan MAT 252; Finnigan MAT, Bremen, Germany). For determination of plasma palmitate, FFAs were extracted from plasma, isolated by thin-layer chromatography, and derivated to their methyl esters. Palmitate concentration was determined on an analytical GC with flame ionization detection using heptadecanoic acid as internal standard, and on average, it comprised 23 ± 4% of total FFAs. Isotope tracer/tracee ratio (TTR) of palmitate was determined using GC-combustion-IRMS (Finnigan MAT 252) and corrected for the extra methyl group in its derivative.

Calculations. Isotopic enrichment is expressed as the TTR: $(^{13}C/^{12}C)_{sa} - (^{13}C/^{12}C)_{bk}$, where sa is sample and bk is background. Fractional recovery of label in breath CO_2, derived from the infusion of labeled acetate, was calculated as follows: fractional recovery of label (ar, %) = $(TTRCO_2 \times VCO_2)/(F) \times 100\%$, where $TTRCO_2$ is TTR in breath CO_2, VCO_2 is carbon dioxide production (mmol/min) and F is infusion rate (mmol/min). The rate of [U-^{13}C]palmitate oxidation was calculated as follows: palmitate oxidation (μmol/min) = $(TTRCO_2 \times VCO_2)/(TTR_p \times ar) \times 1{,}000$, where TTR_p is the TTR of fatty acid carbon in plasma and ar is the fractional acetate recovery.

From palmitate oxidation, plasma-derived fatty acid oxidation was then calculated by dividing palmitate oxidation rate by the fractional contribution of palmitate to the total FFA concentration. IMTG- and/or VLDL-derived fatty acid oxidation was calculated by subtracting plasma-derived fatty acid oxidation from total fatty acid oxidation. The latter was determined by converting the rate of total fat oxidation to its molar equivalent, with the assumption that the average molecular weight of triglyceride is 860 g/mol, and multiplying the molar rate of triglyceride oxidation by 3, because each molecule contains 3 mol fatty acid.

Total carbohydrate and fat oxidation were calculated from the measured VO_2 (l/min) and VCO_2 (l/min) using the nonprotein stoichiometric equations (33): total fat oxidation (g/min) = $1.695\ VO_2 - 1.701\ VCO_2$; total carbohydrate oxidation (g/min) = $4.585\ VCO_2 - 3.226\ VO_2$.

Rate of appearance (R_a, μmol/min) of palmitate in plasma, which under steady-state conditions is equal to the rate of disappearance (R_d) minus tracer infusion rate, was calculated as $R_a = F \times (TTR_i/TTR_p)$, where TTR_i is the TTR of fatty acid carbon in infusion. Percentage of plasma FFA cleared from the circulation that was oxidized (percent R_a oxidized) was calculated as percent R_a oxidized = plasma-derived FFA oxidation/R_a FFA.

Statistical analysis. All data are presented as means ± SE, and $P < 0.05$ is considered as the significance level. Differences in measured variables before and after training were tested using paired *t* tests. Repeated measures one-way ANOVA were used to detect differences in variables in time. When significant differences were found, a Scheffe's post hoc test was used to determine the exact location of the difference. For testing differences in blood parameters between treatments, areas under the concentration versus time curve where calculated for 0–120 min at rest and 150–180 during exercise.

RESULTS

On average, subjects completed a total of 31 ± 1.5 sessions during the 12-week training program, with an average duration of sessions of 47.5 ± 2.5 min (5 kcal/kg fat-free mass). Therefore, the average exercise duration per week was 2.0 ± 0.1 h/week. Body weight tended to be lower after the training program ($P = 0.07$) (Table 1). The 12-week training program had no influence on percentage body fat or VO_{2max} (Table 1).

Effect of endurance training on substrate oxidation. [^{13}C]palmitate enrichment was not significantly different between the separate time points at rest (100, 110, and 120 min) and during exercise (160, 170, and 180 min), whereas breath $^{13}CO_2$ enrichment gradually increased due to an increased recovery of ^{13}C label from the TCA cycle exchange reactions (25) (Table 2). At rest, total fat oxidation was not significantly influenced by the 12-week training program (194 ± 18 vs. 210 ± 20 μmol/min before and after training, respectively, $P = 0.62$). Similarly, plasma-derived fatty acid oxidation was not significantly influenced by the 12-week training program (233 ± 24 vs. 197 ± 28 μmol/min before and after training, respectively, $P = 0.31$). However, IMTG- and/or VLDL-derived fatty acid oxidation was significantly higher after the training period (−38 ± 23 vs. 14 ± 25 μmol/min, $P = 0.022$). Because the absolute rate of IMTG- and/or VLDL-derived fatty acid oxidation (which is defined as the difference between total fat oxidation, as measured by indirect calorimetry, and plasma-derived fatty acid oxidation, as measured by stable isotopes) in the resting state is negative, no conclusion can be drawn about the absolute contribution of IMTG- and/or VLDL-derived fatty acid oxidation to total fat oxidation at rest. A negative IMTG- and/or VLDL-derived fatty acid oxidation at rest has repeatedly been found in previous studies (4,19,34) and is probably due to the combination of the different methods to determine fat oxidation. Nevertheless, an increase in the value of IMTG- and/or VLDL-derived fatty acid oxidation after training does indicate that endurance training increases the capacity to oxidize IMTG- and/or VLDL-derived fatty acids at rest.

During exercise, total fat oxidation tended to be higher (29%) after the training program (1,231 ± 94 vs. 1,586 ±

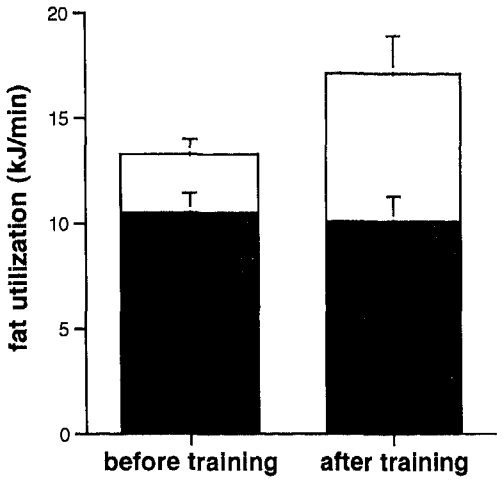

FIG. 1. Relative contribution of plasma fatty acid and IMTG- and/or VLDL-derived fatty acid oxidation to total fat oxidation (kJ/min) before and after the training program. ■, plasma fatty acid oxidation; □, IMTG- and/or VLDL-derived fatty acid oxidation.

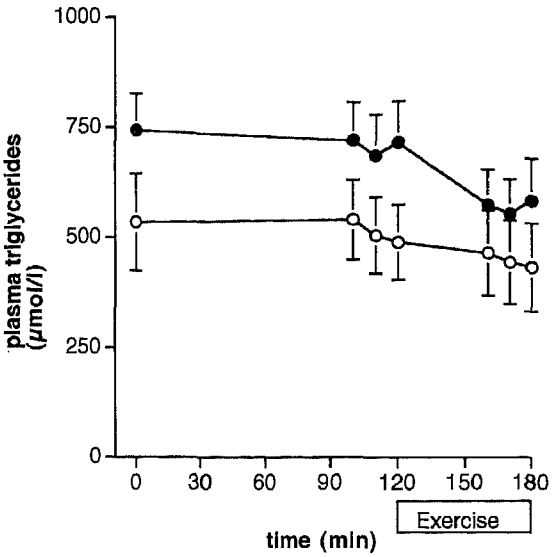

FIG. 2. Plasma triglyceride concentration (μmol/l) measured during 2 h rest and 1 h exercise, before and after the training program. ●, before training; ○, after training.

126 μmol/min, $P = 0.06$) (Fig. 1). Plasma-derived fatty acid oxidation during exercise was not significantly influenced by the training program (972 ± 88 vs. 935 ± 112 μmol/min, $P = 0.80$), but IMTG- and/or VLDL-derived fatty acid oxidation tended to be higher (152%) after training (259 ± 67 vs. 652 ± 167 μmol/l, $P = 0.09$) (Fig. 1). Before the training program, IMTG- and/or VLDL-derived fatty acid oxidation during exercise accounted for 21 ± 5% of total fat oxidation, whereas this almost doubled after the training program (40 ± 8%). Rate of appearance of FFA was not influenced by the training program, neither at rest (580 ± 41 vs. 510 ± 67 μmol/min, $P = 0.37$) nor during exercise (1,167 ± 60 vs. 1,090 ± 157 μmol/min, $P = 0.66$). The percentage of R_a that was oxidized was also not influenced by the training program, neither at rest (40 ± 4 vs. 39 ± 2%, $P = 0.71$) nor during exercise (83 ± 4 vs. 87 ± 3%, $P = 0.45$). At rest, carbohydrate oxidation was not significantly affected by the training program (153 ± 9 vs. 142 ± 14 mg/min before and after training, respectively, $P = 0.56$). Carbohydrate oxidation during exercise tended to be lower after training (1,347 ± 101 vs. 1,115 ± 98 mg/min, $P = 0.09$). Energy expenditure, both at rest (4.63 ± 0.15 vs. 4.62 ± 0.20 kJ/min, $P = 0.98$) and during exercise (35.3 ± 1.60 vs. 35.6 ± 1.96 kJ/min, $P = 0.66$) was not significantly different before and after the training period. Acetate recovery, both at rest (19.4 ± 0.9 vs. 18.4 ± 1.3%, $P = 0.61$) and during exercise (67.2 ± 2.4 vs. 63.4 ± 3.1%, $P = 0.39$) was not different before and after the training period.

Plasma triglyceride concentrations (Fig. 2) were significantly lower after the training period at time zero ($P = 0.011$), at the end of the infusion period (120 min, $P = 0.042$), and tended to be lower at the end of the exercise test ($P = 0.06$). The area under the time versus concentration curve was significantly lower after training ($P = 0.03$). Both at rest and during exercise, the average concentrations for plasma glucose (at rest: 4.91 ± 0.07 vs. 4.86 ± 0.13 mmol/l, $P = 0.50$; during exercise: 4.86 ± 0.14 vs. 4.88 ± 0.13 mmol/l, $P = 0.89$), plasma FFAs (at rest: 574 ± 49 vs. 531 ± 69 μmol/l, $P = 0.51$; during exercise: 693 ± 48 vs. 713 ± 87 μmol/l, $P = 0.80$), and plasma glycerol (at rest: 53 ± 6 vs. 49 ± 6 μmol/l, $P = 0.50$; during exercise: 153 ± 19 vs. 176 ± 21 μmol/l, $P = 0.39$) were not significantly influenced by the training program.

Effect of endurance training on mRNA expression. The 12-week training program had no effect on two genes involved in the transport and oxidation of blood glucose: hexokinase II (2.7 ± 0.7 vs. 2.9 ± 0.4 amol/μg RNA before and after training, respectively, $P = 0.84$) and GLUT4 (43.1 ± 4.3 vs. 37.8 ± 2.0 amol/μg RNA before and after training, respectively, $P = 0.65$). However, the expression of two genes encoding for key enzymes in fatty acid metabolism were affected by the training program: skeletal muscle ACC2 was significantly lower after training (108 ± 24 vs. 69 ± 24 amol/μg RNA, $P = 0.005$) (Fig. 3*A*), whereas there was a tendency for an increased expression of LPL mRNA (45.2 ± 3.4 vs. 88.5 ± 20.0 amol/μg RNA, $P = 0.07$) (Fig. 3*B*). The expression of UCP3 (12.1 ± 3.1 vs. 9.7 ± 2.3 amol/μg RNA, $P = 0.57$) was not influenced by the 12-week training period.

DISCUSSION

The effect of endurance training on the contribution of different fat sources to total fat oxidation after endurance training is under debate. Some studies found that IMTG- and/or VLDL-derived fatty acids contribute highly to the increased fat oxidation after endurance training (12,14–16), whereas others did not observe an effect of endurance training on the oxidation of IMTG- and/or VLDL-derived fatty acids (17,18). Part of this controversy could be explained by the methodological difficulties in using [^{13}C]- and [^{14}C]-fatty acid tracers to estimate the oxidation of plasma fatty acids, especially in the resting state (35). However, Sidossis et al. (19) showed that an acetate recovery factor could be applied to overcome these difficulties. We showed that this acetate recovery is reproducible (25) but has a high interindividual variation and is influenced by infusion period, metabolic rate, respiratory quotient, and body composition (21) and therefore needs to be determined in every individual under similar conditions and at similar time points as the measurement of

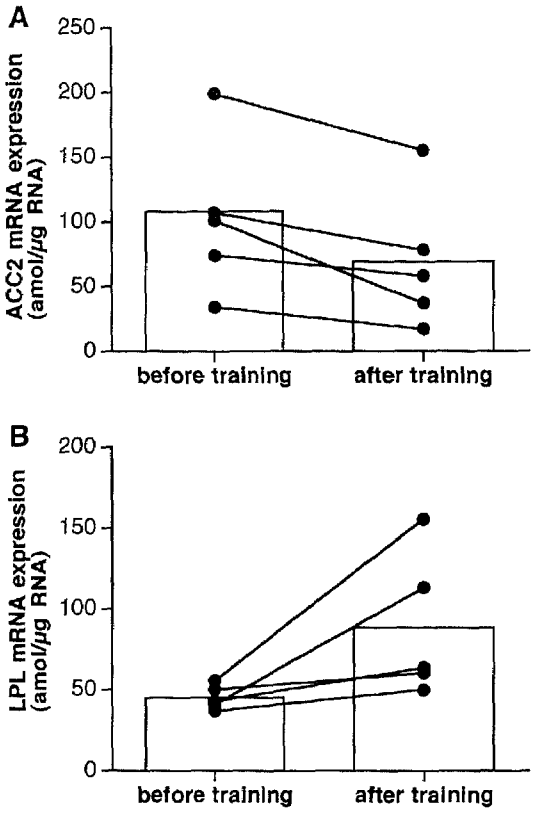

FIG. 3. Skeletal muscle mRNA expression of ACC2 (*A*) and LPL (*B*) (amol/μg RNA) measured after an overnight fast, before and after the training program.

plasma-derived fatty acid oxidation. In the present study, we therefore measured the acetate recovery factor at all time points in each individual both before and after the training program (at least 7 days separated from the last training session to exclude the influence of the last exercise bout on the measurements) and were therefore able to correct plasma-derived fatty acid oxidation rate for loss of label in the TCA cycle. To our knowledge, this is the first study using this validated methodology that clearly shows that the training-induced increase in fat oxidation during exercise is almost completely accounted for by an increase in IMTG- and/or VLDL-derived fatty acid oxidation.

The finding that training (tends to) specifically increase the capacity to oxidize IMTG- and/or VLDL-derived fatty acids both at rest and during exercise is of particular interest. High concentrations of IMTGs are associated with insulin resistance (7,8); therefore, increased rates of IMTG oxidation may be beneficial in the prevention/treatment of type 2 diabetes. With the available stable isotope tracer methodology, we cannot distinguish between IMTG- or VLDL-derived fatty acid oxidation. Using electron microscopy, it has previously been shown that endurance-trained athletes have increased IMTG concentrations (36), and because endurance athletes have an increased fat oxidation capacity, it seems logical that this increased IMTG storage after endurance training is an adaptation mechanism to allow IMTG oxidation during exercise. The localization of the IMTG near the mitochondria would make these triglyceride pools an efficient source of substrate, especially during exercise. However, biochemical analysis of IMTGs is problematic, and therefore the use of IMTG remains controversial. On the other hand, the contribution of VLDL-derived fatty acids to fat oxidation during exercise is also still under debate (18,37). The increased expression of LPL mRNA after training, as observed in our study, which is in accordance with previous studies showing increased LPL activity after endurance training in rodents (38,39), and the reduced plasma triglyceride levels after the training program suggest that VLDL-derived fatty acids contribute significantly to total fat oxidation. Alternatively, an increase in LPL after training might serve to provide fatty acids for the replenishment of IMTGs that have been oxidized during exercise (40). Certainly, further studies are needed to clarify the contribution of IMTG- and VLDL-derived fatty acid oxidation to total fat oxidation.

Another important aspect of the present study is that we have examined the effect of a low-intensity training program for only 2 h per week. Because endurance training has been shown to increase the capacity to oxidize fatty acids, it has been proposed to be beneficial in overcoming the disturbances in fat oxidation often observed in obesity and diabetes (9). However, most of the studies examining the effect of endurance training on fat oxidation capacity have implicated high-intensity exercise (>60% Vo_{2max}) for many hours per week, and these training protocols are not easily incorporated in the daily life of most people. In the present study, the tendency of increased rate of fat oxidation and the marked increase in IMTG- and/or VLDL-derived fatty acid oxidation was achieved with very mild exercise (on average 2 h/week for 3 months). We have chosen this exercise program such that it would be possible to incorporate it in most people's lifestyle, making it applicable for the prevention and/or treatment of obesity and type 2 diabetes. Although we did not examine obese and/or diabetic subjects, it is important to note that, with the same training program, we also found an increase in fat oxidation in obese subjects (34,41).

To investigate the mechanisms behind the changes in substrate oxidation after the endurance-training program, we measured mRNA levels of several genes involved in glucose and fatty acid metabolism. A muscle biopsy was taken 6–7 days before the training program and 6–7 days after the last training session to exclude the influence of acute exercise on mRNA expression. The expression of two genes involved in regulatory steps of glucose metabolism, i.e., GLUT4, the major glucose transporter in skeletal muscle and hexokinase II, which catalyzes the phosphorylation of glucose to glucose-6-phosphate, a possible rate-limiting step for glucose utilization, were not altered by the 12-week training program, showing that low-intensity endurance training mainly affects fatty acid metabolism. As mentioned above, mRNA expression of LPL, which hydrolyzes plasma triglycerides and directs the released FFAs into the tissue (22), tended to increase after training, suggesting that the capacity of skeletal muscle to hydrolyze VLDL triglycerides may be improved by the training program. Inside the muscle cell, ACC2 activity has recently been suggested to control the rate of fatty acid oxidation and triglyceride storage (23). ACC2 catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, an intermediate that inhibits the activity of CPT1. CPT1 catalyzes the rate-limiting step in the transfer of fatty

acyl-CoA into mitochondria, where they undergo oxidation. ACC2 knockout mice were recently shown to have 30% higher fat oxidation rates and to have much lower fat stores compared with wild-type mice (23). Interestingly, ACC2 was decreased by 36% after the training period in our subjects. Although we were not able to measure ACC2 enzyme activity, it is tempting to speculate that a decrease in ACC2 activity after training was responsible for the observed training-induced increase in fat oxidation. Because high levels of malonyl-CoA have been associated with insulin resistance (42), the reduction of ACC2 with endurance training could possibly be beneficial in the treatment of type 2 diabetes. Finally, we determined the expression of the human UCP3, which has recently also been implicated in the transport of fatty acids across the inner mitochondrial membrane (43). In a cross-sectional study, we have previously found that UCP3 mRNA was lower in trained than in untrained subjects (44). In the present study, we did not find a significant effect of the training program on UCP3 mRNA expression, suggesting that the training program was not severe enough to result in changes in UCP3 mRNA. Remarkably, we recently found that, in the same study, UCP3 protein content was significantly decreased after training in all subjects (24). The reason for the discrepancy between the effect of training on UCP3 mRNA expression and protein cannot be deduced from the present study but might involve post-translational regulation, although the number of subjects is too limited to make such a conclusion.

In conclusion, the present study shows for the first time that a low-intensity training program for, on average, 2 h/week leads to an increase in the capacity to oxidize IMTG-and/or VLDL-derived fatty acids at rest. During exercise, total fat oxidation was also increased, and this was mainly accounted for by an increase in IMTG- and/or VLDL-derived fatty acid oxidation. The mechanism behind this adaptation seems to involve a chronic upregulation of LPL mRNA expression and a chronic downregulation of ACC2, potentially leading to lower malonyl-CoA concentration and less inhibition of CPT1. In contrast to moderate- to high-intensity endurance training, the mild training protocol did not increase hexokinase II and GLUT4 expression, indicating that specifically fat oxidation was improved.

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